SYNTHESIS AND STUDIES ON NOVEL REAGENTS FOR THE SPECTROPHOTOMETRIC DETERMINATION OF SOME BIOLOGICALLY SIGNIFICANT METAL IONS AND ANIONS

SYNOPSIS

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SYNOPSIS

SYNTHESIS AND STUDIES ON NOVEL REAGENTS FOR THE SPECTROPHOTOMETRIC DETERMINATION OF SOME BIOLOGICALLY SIGNIFICANT METAL IONS AND ANIONS

Introduction

Analytical chemistry is concerned with providing qualitative and quantitative information about the chemical and structural composition of matter. A huge variety of samples from high concentrations to parts per billion levels of matter in various sources like biological tissues, food samples and water bodies are analyzed by the analyst. In the past few decades, several elegant instrumental techniques like UV-Vis, Fluorescence, AAS, ICP, Mass spectroscopy were frequently employed which are reliable, selective and having a high degree of accuracy. Among these, spectrophotometry and spectrofluorimetry are the two important methods that are widely used for a wide variety of reasons. High accuracy, precision, sensitivity and ease of availability made this technique indispensable for modern analytical chemists [1, 2].

The development of optical metal ion and anion chemosensors with high selectivity and sensitivity is essential as these inorganic ions participate or are responsible for some biological and chemical processes. The sensor is the system that undergoes a change in its own state, its characteristics by the stimulation of any form of energy leading in the qualitative and quantitative analysis of stimulant. The photophysical and optical changes in a molecule are found more important in this regard. These chemosensors exhibit sensitivity and selectivity to specific metal ions and anions. Ionic dipole interactions, electrostatic interactions, π-π^* interactions and H-bonding are the main driving force for the interaction of various ions with organic compounds, these are the main controlling factor in the field of supramolecular chemistry. These factors are led to the design and development of host-guest complexes and getting selective chemosensors (host) for the recognition of various analytes (guests) [3].

When it comes to the qualitative and quantitative recognition of metal ions and anions, the essential prerequisite is chemosensors which can be used for the analyte of interest. On the other hand, metal and anion recognition is very important because many of these

play a crucial role in physiological functions and also some are the prospective reason for environmental pollution [4].

Fluoride (F^-) is the most electronegative atom with distinct chemical properties with its ability to form a strong hydrogen bond. It is important for dental care and hence it is used in all oral care items like mouth wash and toothpaste and also it helps in maintaining healthy bone structures in the human body. However, excess intake of fluoride ions results in a disease called skeletal fluorosis that causes stiffness of bones, pain in the joints and also calcification of bones. The rate of fluoride ion absorption and excretion in the human body is much varied as it is absorbed easily but excreted slowly. Due to these many health reasons, the addition of fluoride more than 0.5-1.0 mg L^{-1} in drinking water and 1000 mg L^{-1} in oral care products lead to harmful effects [5]. The bioaccumulation of nitrogenous pollutants like **nitrite (NO_2^-) and nitrate (NO_3^-)** in water and soil poses a hazard to humans and plants. Nitrite is also utilized as an additive agent in the food industry and its mishandle might lead to an excessive amount of nitrite in consumables which is unwholesome to public health. Organic nitrogen undergoes biochemical oxidation to form nitrate and can be readily converted into the more toxic nitrite by microbial reduction. The WHO recommends 6.5×10^{-5} M is to be the maximal allowed nitrite concentration in drinking water [6, 7]. **Mercury (Hg^{2+})** is the most prevalent toxic metal ion, even though it plays some role in different areas. It can be classified into three main groups, namely, metal (Hg), organic mercury (CH_3Hg^+), and inorganic mercury (Hg^{2+}). Although mercury is being used in electronics, cosmetics, and batteries, improper disposal of inorganic mercury could cause detrimental effects on the environment. According to the US Environmental Agency and WHO, the permissible amount of mercury in drinking water is 2 and 6 µg L^{-1}. As per the US Food and Drug Administration, the maximum allowable seafood concentration is 1 mg L^{-1}. Further, different kinds of microorganisms like sulfate-reducing bacteria can produce methyl mercury from its inorganic form, which can cause severe health issues to human beings, including neurological problems, kidney abnormality, myocardial infarction, Minamata, and some type of autism by distracting the immune, endocrine, and central nervous systems when it is accumulated in the body. Contamination of water sources, food sources with mercury is alarming because it remains as such in the environment and eventually enters and accumulates in the food chain [8, 9]. Being the third most abundant metal ion in the human body (after iron and zinc), copper **(Cu^{2+})** plays an important role

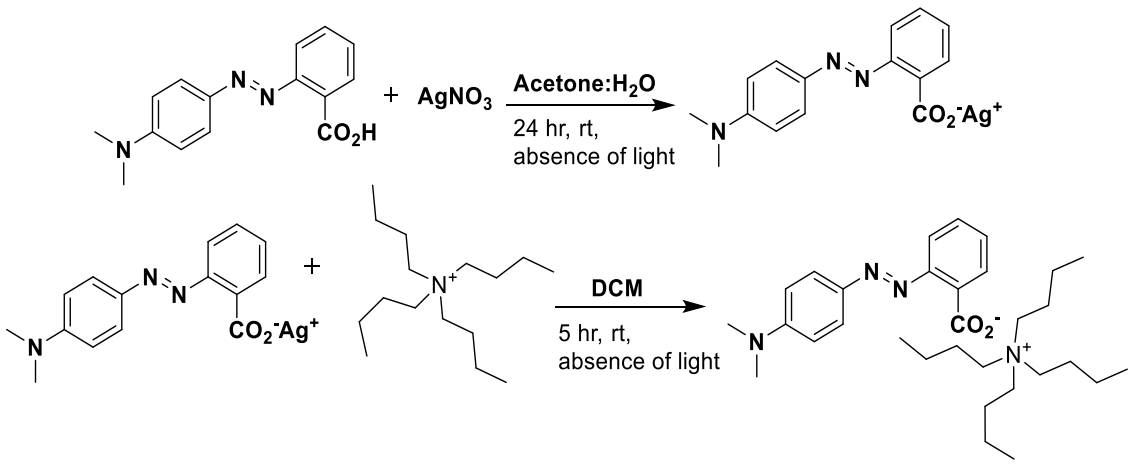
in physiological processes. It is a cofactor of several key enzymes like tyrosinase, cytochrome co-oxidase and superoxide dismutase and it is also involved in energy generation, signal transduction and dioxygen activation and transport. Since the thermal and electrical conductivity of Cu^{2+} is high, it is being used as an electrical conductor and thermal conductor and also it is essential for the preparation of some metal alloys. Due to its antifungal nature, it is used as an antifungal agent in areca, pepper and some other crops. These various applications are led to the release of more and more Cu^{2+} to the environment. This is detrimental to the human body, leads to various disorders such as Parkinson's disease, Alzheimer's disease, Wilson's disease, lipid metabolism disorder, Menkes syndrome and prion disease, WHO limits the amount of Cu^{2+} in drinking water to 20 μM. Therefore, the detection of Cu^{2+} has remained important as far as the environment and health are concerned [10, 11]. **Aluminium (Al^{3+})** is the most abundant metallic element within the lithosphere and the third most abundant on Earth. It can be a potential toxicant and its significant accumulation in the human body has been related to a wide range of diseases like bone softening, anaemia or dialysis encephalopathy. It is also a well-known neurotoxic and overexposure leads to an increased risk of cognitive disorders, Alzheimer's and Parkinson's diseases. The adverse effects affect especially patients with a renal disease requiring dialysis, preterm infants and young children. The World Health Organization has established a provisional tolerable weekly intake (PTWI) of 7 mg kg^{-1} body weight as aluminium from all sources (20 mg/person/day for a 70 kg adult) [12, 13].

Within this context, the development of novel chemosensors for continuous monitoring of these anions in various physiological and environmental samples is of extreme prominence and has become an active research area. However, the development of selective and sensitive chemosensors for metal/anion recognition is a challenging task. A review of the literature revealed that, during past decades, substantial research has been focused on the development of molecular receptors for many analytes such as fluoride, nitrite, copper, aluminium and mercury because of their important role in biological systems and environmental pollution. Various analytical methods, such as a chromatographic, ion-selective electrode, fluorometric, colorimetric, flow injection and electrochemical analyses have been developed for the sensing of metal ions and anions. Among the various receptors, colorimetric and fluorimetric type presents many advantages, including high sensitivity, low cost, easy detection by the naked eye without

any expensive instruments and suitability as a diagnostic tool for biological concerns. Therefore, it is high time to develop sensitive, selective and quick time sensing receptors for such toxic anions. Despite a large number of different receptor molecules studied and described so far in the literature, few systems display selectivity and affinity, easily adaptable to practical application, especially in water. The present investigation entitled '**Synthesis and Studies on Novel Reagents for the Spectrophotometric Determination of Some Biologically Significant Metal ions and Anions**' has been discussed in seven chapters. A short description of the contents details in each chapter is described below.

Chapter 1 is an introduction to the entire work which elucidates an overview of ion sensors and general analytical techniques employed in the analysis and detection of various metal ions and anions with the importance of UV-Visible and fluorescence measurements. It also gives a brief outline of the selection of ions and chromogenic reagents used in the present investigation for the quantitative/qualitative determination of metal ions and anions.

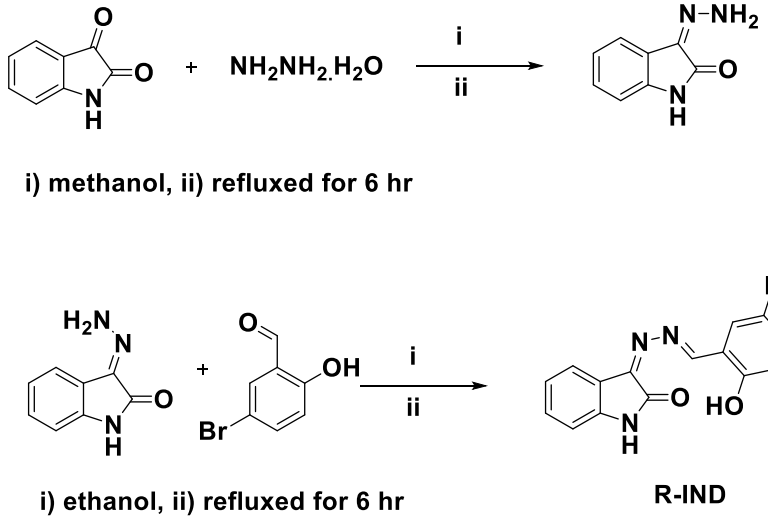
Chapter 2 describes an approach for the determination of nitrite using tetrabutylammonium modified methyl red ([TBA]MR) ionic liquid in water samples and farm products. The synthesis of [TBA]MR is shown in **scheme 2.** Nitrite initiates [TBA]MR to undergoes oxidative degradation in acidic media with potassium bromate. Under optimum conditions, linearity was obtained for the nitrite concentration in the range of 0.05-14 µM with the correlation coefficient, LOD and LOQ of 0.9932, 0.046 µM and 0.138 µM respectively. Compared with the other method of the same kind, 10 times better in terms of time with a good detection limit was obtained. The activation energy of the catalytic reaction is 60.22 KJ mol^{-1}. Moreover, the proposed method has been applied to detect nitrite levels in water samples, farm products with good recoveries.



Scheme 2. Synthesis of [TBA]MR

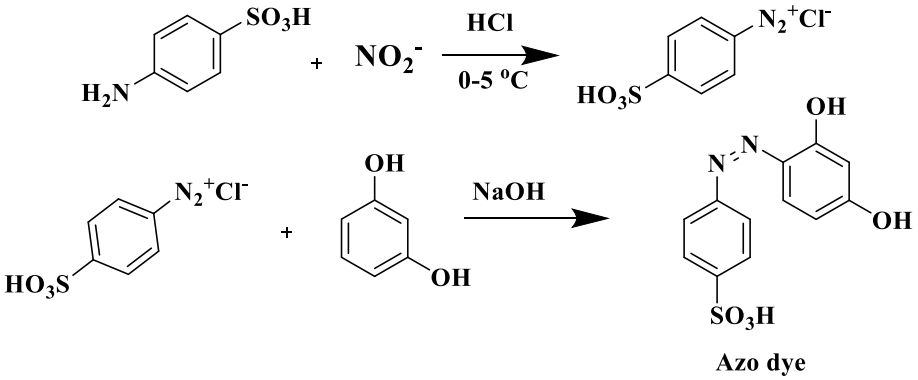
Chapter 3 briefs about a new colorimetric sensor 3-((-5-bromo-2-hydroxybenzylidene)hydrazono)indolin-2-one (R-IND) based on isatin hydrazones, designed and synthesized by a simple Schiff base reaction for the determination of F^- ion as shown in **Scheme 3.** The anion sensing properties of R-IND were examined, characterized by common spectroscopic techniques such as Fourier Transform-Infrared (FT-IR), Ultraviolet-Visible (UV-Visible), ^1H and ^{13}C NMR, Electrospray Ionization Mass Spectroscopy (ESI-MS. The sensor R-IND bearing ortho hydroxy groups acting as binding sites. The sensor R-IND exhibited sensitive and selective sensing towards F^- ion over the other anions like Cl^-, I^-, Br^-, $H_2PO_4^-$, AcO^-, CN^-, NO_3^-, ClO_4^- and HSO_4^- in ACN and ACN:H_2O (8:2, v/v) media. The anion-free solution of R-IND has shown an intense absorption band at 328 nm. Upon addition of F^- ion of certain amounts, the strong absorption band at 328 nm was shifted to the higher wavelength of 550 nm and 556 nm in ACN and ACN:H_2O (8:2, v/v) media respectively, whereas the addition of other anions did not alter the absorption peak indicating that other than F^- ion does not affect the UV-Vis spectra of R-IND. This shift in the absorption band was induced by the F^- ion, which is also called a bathochromic shift or redshift. This bathochromic shift of 222 and 228 nm can be attributed to the removal of the hydrogen from the hydroxy groups of host moiety (R-IND) by the guest F^- ion leading to the formation of hydrogen fluoride. This was further accounted by the ^1H NMR titration experiments. The Job's plot analysis reveals 1:2 stoichiometry between the host-guest complex. The binding constant was found to be $1.72\times10^9\ M^{-1}$ and $2.82\times10^9\ M^{-1}$ in ACN and ACN:H_2O (8:2, v/v) respectively

which was calculated using a B-H plot. The detection limit of sensor R-IND was turned out to be 0.45 and 0.41 ppm for F^- ion in organic and aqueous organic medium which is lesser than World Health Organization (WHO) permission level (1 ppm). Finally, economically viable paper-based colorimetric test strips of R-IND were developed to detect the F^- ions in an aqueous solution.



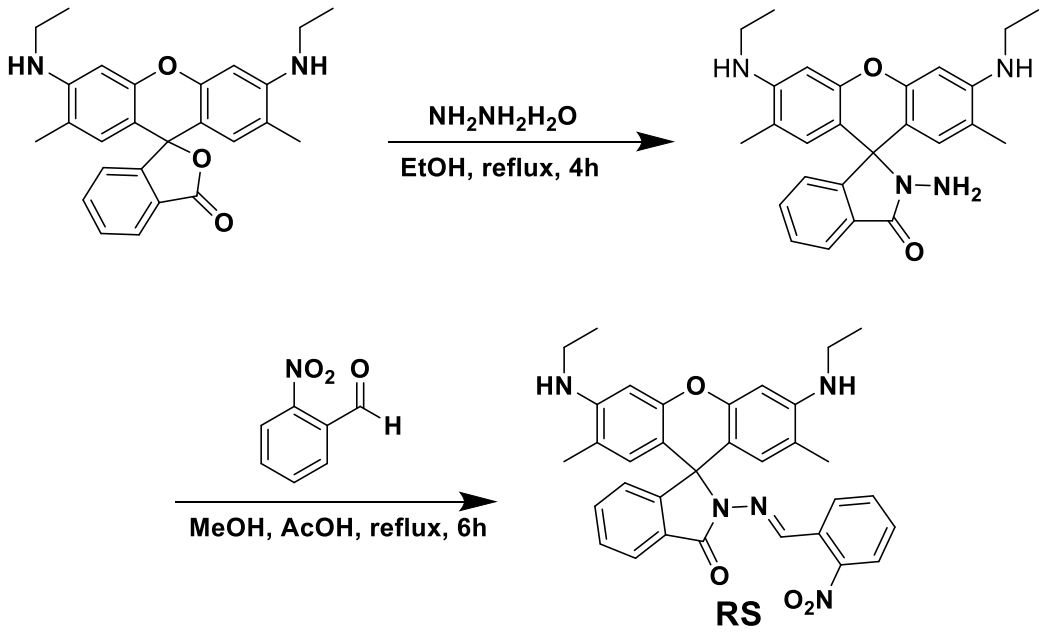
Scheme 3. Synthesis of sensor R-IND

Chapter 4 depicts an efficient method developed for the colorimetric determination of nitrite and nitrate ions in water and soil samples by in-situ formation of azo dye as shown in **scheme 4** which showed λ_{max} at 385 nm in UV-Vis spectrum. The reaction condition and the concentration of reagents used are optimized. The molar absorptivity, Sandell's sensitivity, detection limit and quantification limit of the method are found to be 3.22×10^4 L mol^{-1} cm^{-1}, 1.98×10^{-6} µg cm^{-2}, 0.0030 µg mL^{-1} and 0.0092 µg mL^{-1} respectively with the linearity range up to 2.6 µg mL^{-1}. In order to reduce the organic load in the test samples, the azo dye formed during the determination of nitrite and nitrate was successfully removed by the custom-made eco-friendly cellulose-modified hydrogel. The structure, morphology and thermal properties of the hydrogel are determined by Fourier-Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and Thermogravimetric Analysis (TGA) respectively. The dye removal mechanism involves pseudo-second-order kinetics, adsorption was found to be spontaneous based on the thermodynamic parameters and it was befitting to the Freundlich isotherm model.



Scheme 4. Synthesis and formation of azo dye

Chapter 5 describes the design and development of a colorimetric/fluorometric rhodamine & 2-nitrobenzaldehyde based Schiff base chemosensor 3',6'-bis(ethylamino)-2',7'-dimethyl-2-((2-nitrobenzylidene)amino)spiro[isoindoline-1,9'-xanthen]-3-one (RS) derived by simple two steps condensation employing ethanol as a solvent for the detection of Al^{3+} and Hg^{2+} ions and the synthetic route have been shown in **scheme 5**. The molecular structure of the sensor RS was confirmed by Single-crystal X-ray analysis. A neat metallic red color single crystal suitable for X-ray Diffraction was developed by slow evaporation using ACN at room temperature. An ORTEP view of RS with atomic labeling is shown in **Fig. 1.** The metal ion sensing ability of sensor RS has been explored and the results revealed that RS is capable of detecting Hg^{2+} and Al^{3+} ions over the other competiting metal ions like Ba^{2+}, Cu^{2+}, Cd^{2+}, Fe^{3+}, Fe^{2+}, Mg^{2+}, Mn^{2+}, Pb^{2+}, Ni^{2+}, Zn^{2+}, Na^{+}, K^{+}, Li^{+}, Ag^{+} through UV-Vis and fluorescence spectra with great sensitivity and selectivity. The sensing studies have been performed in ACN:HEPES buffer solution (1:1, v/v) medium and Job's plot revealed that the 1:1 binding stoichiometry between the sensor RS and metal ions. The rapid sensing time of 10 sec along with the UV-Vis and fluorescence spectral changes could be due to the spirolactam ring-opening in the sensor RS followed by the formation of suitable coordinate geometry for Hg^{2+} and Al^{3+} in the backbone of RS. The B-H plot gives the binding constant and it was found to be 4.75×10^{8} M^{-1} and 2.80×10^{9} M^{-1} for Al^{3+} and Hg^{2+} ions respectively. The RS exhibits a quite good detection limit of 1.75 µM and 0.75 µM for Al^{3+} and Hg^{2+} ions respectively. Also, an *in vivo* sensing experiments using *Drosophila melanogaster (Canton-S) and Poecilia reticulate* (Guppy fish) were performed to test its efficacy at identifying Hg^{2+} toxicity within an organism.



Scheme 5. Synthesis of sensor RS

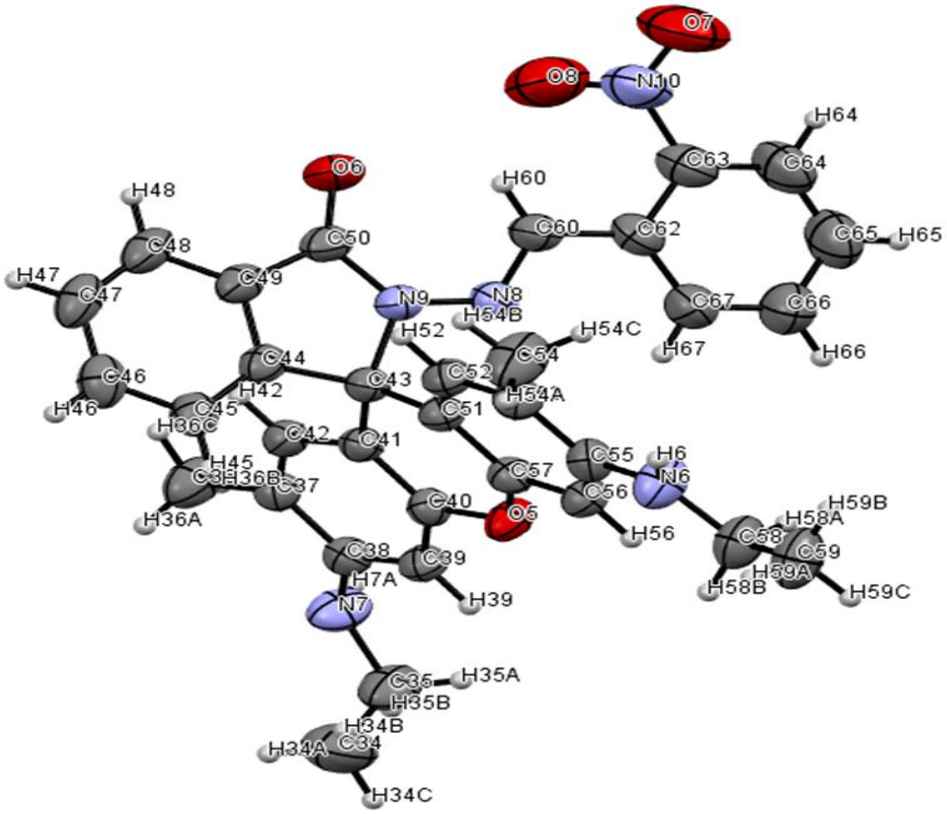
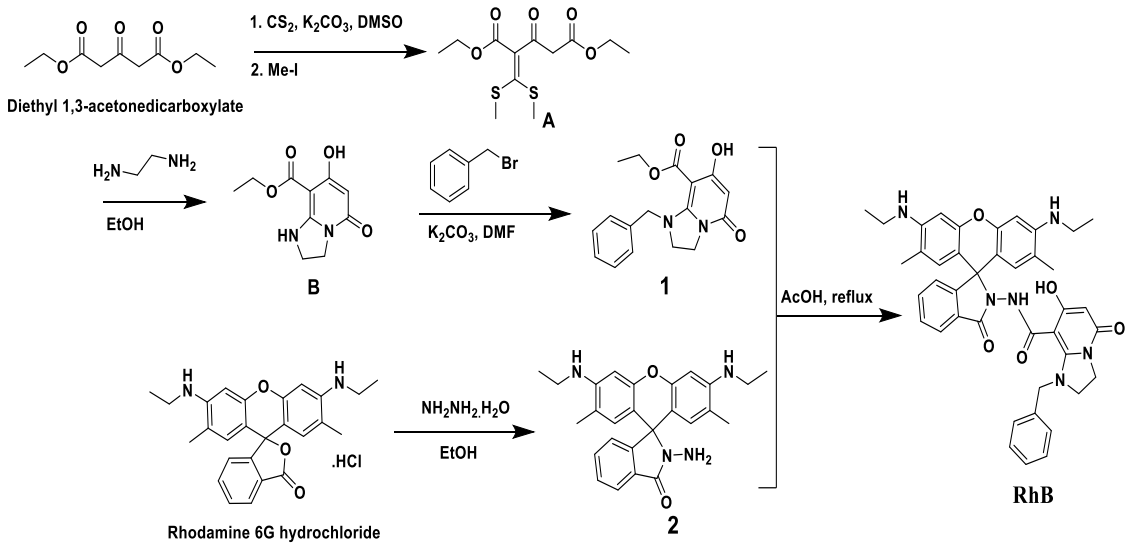


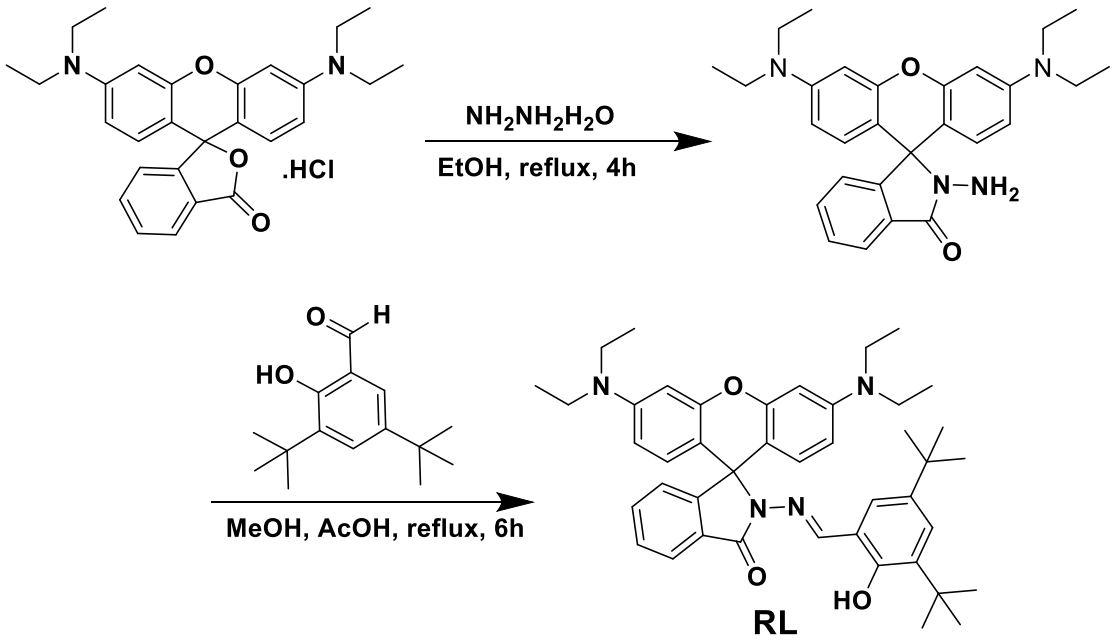
Fig. 1. Molecular structure (ORTEP) of the sensor RS

Chapter 6 depicts the design, synthesis and sensing property of a novel "turn on" fluorimetric sensor 1-benzyl-N-(3',6'-bis(ethylamino)-2',7'-dimethyl-3-oxospiro[isoindoline-1,9'-xanthen]-2-yl)-7-hydroxy-5-oxo-1,2,3,5-tetrahydroimidazo[1,2-a]pyridine-8-carboxamide (RhB) based on rhodamine moiety for the detection of Hg^{2+} ion is as shown in **scheme 6**. The Hg^{2+} sensing ability of RhB was examined and validated using UV-visible, fluorescence and NMR spectral studies. The sensor RhB exhibits selective and sensitive sensing towards Hg^{2+} over the other competitive metal cations like Al^{3+}, Ba^{2+}, Cu^{2+}, Cd^{2+}, Fe^{3+}, Fe^{2+}, Mg^{2+}, Mn^{2+}, Pb^{2+}, Ni^{2+}, Zn^{2+}, Na^{+}, K^{+}, $Li^{+,}$ Ag^{+} in EtOH:HEPES buffer solution (1:1, v/v) medium and a rapid 1:1 stoichiometric irreversible reaction was obtained between RhB and Hg^{2+}. The UV-Vis and fluorescence spectral changes suggest the effectiveness of the sensor and the rapid response of RhB towards Hg^{2+} could be due to the formation of the coordinate bond between the ligand and metal. The spirolactam ring opening followed by the formation of suitable coordinate geometry in the RhB is the reason for the selective sensing of Hg^{2+} ion. This was further confirmed by ^{1}H NMR titration experiments. The binding constant was calculated by the B-H plot and found to be 4.0×10^{9} M^{-1}. The detection limit of the sensor RhB was found to be 2.57 µM and is lesser than the WHO permissible level. The economically feasible, easy-to-use test strips of RhB were developed for the naked-eye detection of Hg^{2+} in an aqueous solution. Also, an *in vivo* sensing experiment was performed using *Drosophila melanogaster (Canton-S)* to test its efficacy at identifying Hg^{2+} toxicity within an organism.



Scheme 6. Synthesis of sensor RhB

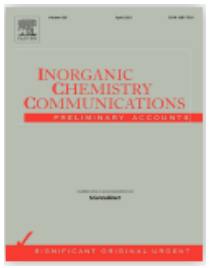
Chapter 7 describes the design and development of a colorimetric/fluorometric rhodamine & 2-hydroxybenzaldehyde based Schiff base chemosensor 2-((3,5-di-tert-butyl-2-hydroxybenzylidene)amino)-3',6'-bis(diethylamino)spiro[isoindoline-1,9'-xanthen]-3-one (RL) derived by simple two steps condensation employing EtOH:HEPES buffer solution as a solvent for the determination of Cu^{2+} ion and the synthetic route has been shown in **scheme 7.** The metal ion sensing ability of sensor RL has been explored and the sensing studies have been performed in EtOH:HEPES buffer solution (1:1, v/v) medium and Job's plot revealed that the 1:1 binding stoichiometry between the sensor RL and Cu^{2+} ion. The rapid sensing time of 10 sec along with the UV-Vis and fluorescence spectral changes could be due to the spirolactam ring-opening in the backbone of RL followed by the formation of suitable coordinate geometry between RL and Cu^{2+} ion. The B-H plot gives the binding constant between the host and guest and it was found to be 5.77×10^{8} M^{-1}. The RL exhibits a quite good detection limit of 1.16 µM. The cost-effective real-time detecting test strips of RL have been developed for the naked eye Cu^{2+} detection in an aqueous solution with visible color changes. Also, an *in vivo* experiment using *Drosophila melanogaster (Canton-S)* was performed to test its efficacy at identifying Cu^{2+} toxicity within an organism.



Scheme 7. Synthesis of sensor RL

List of research papers published and presented in conferences

1. **Gauthama B. U**, B. Narayana, N. K. Suresh, B. K. Sarojini, Y. Sangappa, A. K. Kudva, S. V. Raghu, G. Satyanarayana. Rhodamine-based hybrid moiety for selective colorimetric/fluorimetric "off-on" in field mapping of toxic Hg(II) and its application for in-vivo bioimaging. *Microchemical Journal*, 2021, 166, 106233. **(Impact factor: 4.82)**



2. **Gauthama, B. U**., Narayana, B., Sarojini, B. K., Manjunatha, J. G., & Suresh, N. K. Colorimetric 'naked eye sensor for fluoride ion based on isatin hydrazones via hydrogen bond formation: Design, synthesis and characterization ascertained by Nuclear Magnetic Resonance, Ultraviolet-Visible, Computational and Electrochemical studies. *Inorganic Chemistry Communications*, 2020, 121, 108216. (**Impact factor: 2.49**)

3. **Gauthama, B. U**., Narayana, B., Sarojini, B. K., Bello, K., & Suresh, N. K. Nitrate/Nitrite determination in water and soil samples accompanied by in situ azo dye formation and its removal by superabsorbent cellulose hydrogel. *SN Applied Sciences*, 2020, 2(7), 1-16.

4. **Gauthama B. U**, B. Narayana, B. K. Sarojini and Kabiru Bello. A sensitive spectrophotometric system for the detection of nitrite/nitrate ions in water samples and soil samples. *At 37th annual conference of ICC* organized by NITK Surathkal during 12 - 14 December 2018.
5. **Gauthama B. U**, B. Narayana, B. K. Sarojini and Suresh N Kodlady. Colorimetric, naked eye anion sensors based on isatin hydrazones for recognition of biologically relevant fluoride and cyanide ions in organic medium, insight real-life application and DFT studies. *International Conference on ADVANCES IN CHEMICAL AND MATERIALS SCIENCE*, organized by Mangalore University during October 17-19, 2019.
6. **Gauthama B. U**, B. Narayana, B. K. Sarojini and Suresh N Kodlady. Fluoride and cyanide, dual ion sensor: Design, Synthesis and Characterization ascertained by NMR, UV-Vis and DFT Studies. National Conference on *RECENT NOVEL APPROACH IN CHEMICAL SCIENCE*, organized by FMKMC, Madikeri, FEB 12, 2020

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